

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: William Velander *et al.*

Serial No.: 10/049,849

Group No.: 1632

Filed: 06/27/2002

Examiner: Hama, J.

Entitled: **Transgenic Prothrombin And Related Prothrombin Precursors**

**SECOND DECLARATION OF
DR. WILLIAM VELANDER UNDER 37 CFR § 1.132**

Mail Stop –Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Examiner Hama:

I, Dr. William Velander, under penalty of perjury, state that:

1. I am a co-inventor of the United States patent application captioned above.
2. I am qualified as an expert in the field of protein expression from transgenic mammals.
3. Recent research conducted under my direction has provided data relevant to the above captioned patent application. A transgenic pig (Grandma) was created in accordance with the teachings of the above captioned application that secretes milk having at least 1.5 mg/ml of a human prothrombin amino acid sequence.
4. The prothrombin assay was conducted by first collecting a daily milk sample by inducing lactation. The sample was then processed and diluted by adding aminocaproic acid and ethylene diamine tetraacetic acid. A aliquot of each milk sample collected on Day 5, 10, 15 and 20 following the lactation induction protocol were used to generate a Western Blot immunoassay (nonreduced 12% Bis-Tris Gel) where prothrombin was detected using a horseradish peroxidase

labeled anti-prothrombin polyclonal antibody (US Biologicals, P9115-16A, Lot # L9080754). See, Figure A.

5. Each gel band was analyzed using densitometry using Adobe Photoshope image analysis that measures absolute intensity in terms of "mean x the total number of pixels". These data were compared against a standard curve generated from a parallel analysis of plasma derived factor II/IIa as a standard reference at three different concentrations. See, Figure B. Sample dilution correction factors were determined in terms of the contribution of raw milk in each assayed sample: Day 5: 24.03%; Day 10: 23.46%; Day 15: 23.31%; and Day 20: 23.57%. In other words, the dilution of each raw milk sample was on the order of 1:4.
6. After data interpolation and back calculations accounting for the above sample dilution percentages the final prothrombin concentrations were determined for the following collection days:

Day 5: 1.64 mg/ml (absolute intensity = 515562.80)

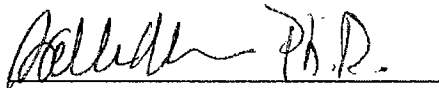
Day 10: 1.67 mg/ml (absolute intensity = 513957.30)

Day 15: 1.11 mg/ml (absolute intensity = 442443.95)

Day 20: 0.79 mg/ml (absolute intensity = 402704.64)

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: October 6, 2010



Dr. William Velander

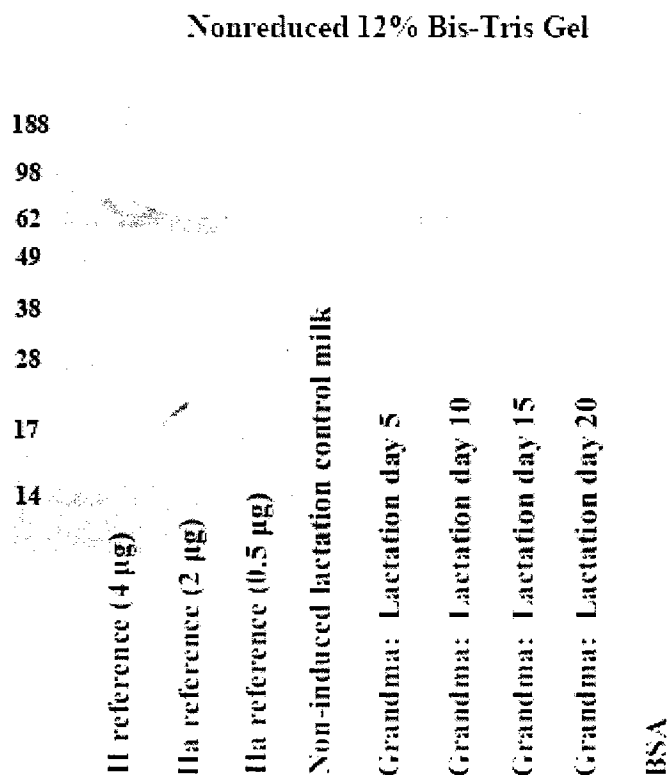


Figure A

Create standard curve:

	µg	Mean Intensity	Std Dev	Pixel	Absolute Intensity
Standard	0.5	84.64	4.89	3.698	312,998.72
	2	106.98	8.67	4.101	438,724.98
	4	115.45	7.36	4.382	505,901.90

$$y = mx + b$$

$$m = 53,951.59$$

$$b = 302,313.43$$

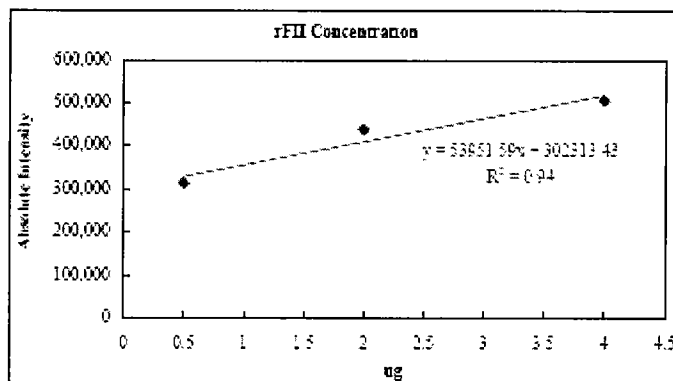


Figure B